

Mycorrhiza and Carbon Flow to the Soil

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SUMMARY. The loss of energy-rich carbon compounds from plant roots to soil microbial populations constitutes a fundamental supply process to the soil ecosystem and the direct supply of host assimilates from mycorrhizal host plants to their fungal symbionts is of significance, not only to the mycorrhizal associations themselves, but also to the soil ecosystem as a whole. The flow of carbon to mycorrhizal roots, and through mycorrhizal mycelia to different components of the soil ecosystem, can clearly be significant, but further information is required about the amounts and types of compounds involved and the mechanisms regulating their translocation and ultimate partitioning. Existing biomass estimates of vesicular-arbuscular and ectomycorrhizal extramatrical mycelium and fruiting structures are considered in relation to theoretical estimates of necessary carbon flow and available experimental estimates, but the lack of adequate methods for quantifying mycorrhizal mycelium remains a barrier to progress in understanding the dynamics of mycorrhizal mycelia and their interactions with other organisms. The flow of carbon into and through mycorrhizal mycelia has a potentially large range of wider effects in the soil ecosystem since energy-rich substrates are required by most biological processes. Potential effects include interactions with phytopathogens and decomposers, chemical defences against grazing of the mycelium, and stabilization of soil aggregates. Energy-rich substrates are also necessary for the synthesis of enzymes to degrade organic polymers and the significance of this enzymatic activity in different environments is discussed in relation to nutrient availability and possible effects on decomposer populations. The formation of mycelial connections between plants and flow of carbon through these may influence plant community development through effects on regeneration processes or plant competition. All of the above effects may have an impact on energy flow and cycling of nutrients.

Introduction

The loss of energy-rich compounds from plant roots to soil microbial populations constitutes one of the fundamental supply processes to the soil ecosystem. Mycor-

rhizal fungi are unique within the soil microbial community in that they have direct access to host assimilates and the supply of energy-rich carbon compounds from mycorrhizal host plants to their fungal symbionts is thus of significance, not only to the mycorrhizal associations themselves, but also the soil ecosystem as a whole.

In this chapter we review existing knowledge concerning the supply of carbon compounds to mycorrhizal roots and mycelium, and their subsequent distribution and cycling within the soil ecosystem. The carbon requirements of different mycorrhizal fungi are discussed and carbon transfer from host root tissue to the fungal biomass is considered in relation to available information on the amounts and types of compounds that are translocated, as well as their ultimate partitioning. Problems of estimating mycorrhizal mycelial biomass are discussed and existing biomass estimates of vesicular-arbuscular (VA) and ectomycorrhizal extramatrical mycelium and fruiting structures are considered in relation to theoretical estimates of necessary carbon flow and available experimental measurements. Regulatory and adaptive processes influencing mycorrhizal mycelia are also discussed.

Finally the flow of carbon compounds into and through mycorrhizal mycelia is considered in relation to its possible wider effects in the soil ecosystem. The supply of energy-rich substrates is essential for synthesis of enzymes to degrade organic polymers and the extent and significance of this enzymic capability in different environments are discussed in relation to nutrient availability and possible effects on microbial decomposer populations. The formation of mycelial connections between plants and flow of carbon compounds through these may influence the source–sink relationships of differently illuminated mycorrhizal plants, with potential effects on regeneration and community development. The possible existence and significance of mycorrhizosphere effects are discussed in connection with processes such as exudation, death, decomposition and recycling of mycelial nutrients, and production of antibiotics. Possible interactions with other microbial populations are discussed.

Carbon Flow from the Host to the Mycobiont

The processes involved in the development of mycorrhizal infection in root systems frequently lead to increased rates of photosynthesis and translocation of carbon compounds to the root systems of host plants. Information concerning the full extent of this translocation, including that to the extramatrical mycelium, the nature of the compounds involved, and the processes regulating their partitioning between different sinks is, however, still limited.

Carbon Requirements of Mycorrhizal Fungi

Different types of mycorrhizal fungi show wide variation in the extent to which they appear able to grow independently of their normal autotrophic hosts. The

Glomeales of vesicular-arbuscular mycorrhizal (VAM) fungi appear to be ecologically obligate symbionts with little or no capacity for independent growth or production of enzymes to degrade complex carbohydrate polymers such as cellulose or pectin. On the other hand, many species of ectomycorrhizal fungi have been isolated and grown on a wide range of culture media. They are generally able to utilize simple sugars such as the monosaccharides glucose, mannose, and fructose, and show intra- and interspecific variation in the degree to which they are able to use disaccharides such as sucrose and trehalose, the simpler oligosaccharides, and compounds such as starch, glycogen, and inulin. Some strains of some species appear to be able to utilize pectic substances to support growth, but the ability of ectomycorrhizal fungi to use complex carbohydrates such as lignin and cellulose is generally considered to be limited. Ericoid mycorrhizal fungi can also be grown on a wide range of carbon compounds such as monosaccharides (including the pentose xylose), disaccharides such as maltose, sucrose, and cellobiose and complex carbohydrates such as pectin and starch. Their cellulolytic activity in pure culture appears to be restricted.

The fungi isolated from orchid roots differ from most other mycorrhizal fungi in that they possess a considerable capacity for independent saprophytic or parasitic growth. In addition to using soluble sugars, most of the tested isolates appear capable of utilizing starch and pectin as well as complex insoluble polymers such as cellulose and some even degrade lignin (Hadley & Ong, 1978). While fungi such as *Rhizoctonia solani* and *Armillaria mellea* may be widespread as parasites, the distribution and importance of these and other fungi as mycorrhizal symbionts represent a more specialised form of association and orchid mycorrhizas are therefore not considered further in this chapter.

The ease with which many ectomycorrhizal fungi can be cultured and the fact that some of them can spread to the rhizosphere of nonhost plants suggest that some may not be obligately symbiotic. However, under natural ecological conditions, the degree of facultative symbiosis may be low and the production of fruit bodies, in some species at least, has been shown to depend on the presence of living host roots (Romell, 1938, 1939). The distribution of many ericoid endophytes also appears to be wider than that of their host plants, suggesting that they may have some ability to survive as soil saprophytes, or as resting structures in the vicinity of roots of nonhost plants.

Pure culture studies do not fully reflect the natural conditions pertaining when growth is in symbiotic association with a host plant. The nutrient concentrations frequently used in pure culture studies are artificially high and the ability or inability of fungi to grow on single carbon sources may not be an accurate reflection of how these are used in situations in which a natural spectrum of compounds is available. Enzymes for the utilization of certain substances may be adaptive and a supplementary carbon source in addition to other nutrients is often needed to allow enzyme synthesis during the period of adaptation. Problems of catabolite repression have often been overlooked and it is important to distin-

guish between production of enzymes for restricted and localized hydrolysis and enzymic activity that is sufficient to support growth using the products as the sole carbon source.

Generalizations from *in vitro* studies of activity must be made with caution. The classical view is that ectomycorrhizal fungi are able to use only simple sugars as carbon sources, but a number of interesting exceptions have been discussed by Lindeberg (1986). There is now a growing consensus that the proteolytic activity of some ecto- and ericoid mycorrhizal fungi may be greater than previously appreciated (Abuzinadah et al., 1986; Abuzinadah & Read, 1989; Leake & Read, 1989) and carbon released from such proteolytic activity may reduce the carbon drain to the symbiont from host plants. The ability of the ericoid endophyte *Hymenoscyphus ericae* to utilize organic acids as carbon sources (Leake, 1988) may also be important in this and other respects. The significance of these findings is complicated by the fact that our knowledge of the availability and dynamics of possible organic nutrients is less well developed than for mineral nutrients.

While the ability to grow mycorrhizal fungi in pure culture is of great practical importance to laboratory experimentation, studies of intact mycorrhizal associations have the advantage in that a more natural carbon balance can be achieved when both symbiotic partners are grown together.

Carbon Translocation to Mycorrhizal Roots

It has been assumed for many years that mycorrhizal fungi obtain their carbon compounds from host plants and that there is a net flow of these from autotroph to heterotroph. No direct evidence of this was available until 1957, when Melin and Nilsson (1957) demonstrated the translocation of ^{14}C -labeled photosynthate to roots and fungal sheaths of *Pinus sylvestris* seedlings infected with the ectomycorrhizal fungi *Scutellinia bovinus* and *Rhizopogon roseolus*. Direct evidence of such translocation was initially more difficult to acquire in VAM systems because of the difficulty of separating the symbionts and the rather scant production of external mycelium in artificial systems. Ho and Trappe (1973) performed the earliest experiments showing transfer of photosynthetically incorporated ^{14}C into external mycelium and spores. In later experiments Bevege et al. (1975) and Cox et al. (1975) demonstrated rapid translocation of photosynthate to infected root systems and subsequent incorporation of labeled carbon into both intracellular hyphae and external mycelium. These initial experiments were followed by many more (see Harley & Smith, 1983) that refined our understanding of the type and relative amounts of different compounds translocated to the fungal symbionts.

Ectomycorrhizas contain fungal-specific carbohydrates such as mannitol, trehalose, and glycogen. The rapid conversion of absorbed plant sugars to metabolic intermediates and fungal storage compounds is one way by which ascomycetous and basidiomycetous fungi are thought to maintain a metabolic sink for photosyn-

thatate derived from their hosts (Lewis & Harley, 1965). Although mannitol and trehalose were originally identified as being important carbon sinks, Söderström et al. (1988) also found the alditols, arabitol and erythritol in the mycelia of *Suillus bovinus*, *Pisolithus tinctorius*, and *Paxillus involutus*.

Ericoid mycorrhizas show the same general carbon incorporation patterns as ectomycorrhizas. Sibley and Read (1974) showed accumulation of ^{14}C into glucose, sucrose, and fructose in nonmycorrhizal roots of *Vaccinium* but in mycorrhizal roots the label was incorporated into the fungal carbohydrates mannitol and trehalose and into polymers of glucose (glycogen) and mannose. These observations are consistent with a general transfer of carbohydrate from the host to the heterotroph, a metabolic sink being maintained by establishment of a concentration gradient in favor of transport to the heterotroph through rapid conversion of plant assimilates to fungal-specific carbohydrates.

There have been few detailed studies of the carbohydrate physiology of VA mycorrhizas. The carbohydrate composition of infected and uninfected roots appears similar, and attempts to detect fungal specific sugars such as the mannitol and trehalose have been largely unsuccessful (Hayman, 1974; Bevege et al., 1975). Lewis (1975) suggested that mannitol was unlikely to be present but that failure to detect trehalose might be due to the low ratio of fungal tissue relative to root tissue. Amijee and Sibley (1987) found only glucose, sucrose, and fructose in mycorrhizal roots of *Allium porrum* infected with *Glomus mosseae* but detected two fungal specific sugars in the external mycelium. Trehalose was found mostly in spores while another unidentified carbohydrate appeared in the mycelium. Lipid synthesis in the fungal component of VA mycorrhizas may have an analogous storage role. Increased total lipid levels in VA mycorrhizal roots of onion, clover, and ryegrass were demonstrated by Cooper and Lösel (1978), although the lipid fractions of infected and uninfected roots did not differ qualitatively. Deposition of lipid droplets and increased amounts of membrane lipids associated with arbuscule formation would contribute to this increase and Cox et al. (1975) demonstrated the incorporation of photosynthetically derived ^{14}C in lipid droplets using autoradiography. Nagy et al. (1980) found increases in the amounts of triglycerides and phospholipids associated with mycorrhizal Citrus roots. Three unidentified fatty acids constituted 31–44% of the total lipids in mycorrhizal roots and were not present in uninfected roots.

Partitioning of Host-Derived Assimilates

Host assimilates transferred to mycorrhizas can be distributed in a number of ways and there has been an increasing number of studies of their partitioning and ultimate distribution, both in experimental microcosms and in natural ecosystems. Experimental study of these processes has been complicated by the difficulty of separating and quantifying mycorrhizal mycelia in natural ecosystems (see below) with the consequence that many field studies have been restricted to fruiting

structures and may thus underestimate the full extent of carbon input to the soil ecosystem. Experimental microcosms facilitate the specific study of mycorrhizal mycelia but are generally restricted to studies of early seedling stages since it is not practical to work with mature trees.

The products of photosynthetic assimilation may be distributed in a number of ways. Mycorrhizal infection often results in increased allocation of C to the root system and this may be incorporated into increased root biomass, increased root respiration, mycelial biomass (both within the root and as external mycelium), and mycelial respiration (internal and external), or lost in the form of exudation from the roots or decomposition and leakage of dying mycorrhizal hyphae. Losses may also occur to mycophagous grazer populations.

Many experiments have failed to quantify one or more of these components. Increases in respiration of symbiotic roots may not be entirely due to the respiration of the symbionts themselves. Pate et al. (1979) found higher rates of CO_2 evolution in host tissue subtending *Rhizobium* nodules than in tissue not associated with nodules, and Cox and Tinker (1976) demonstrated that cells containing arbuscules contained 22 times more cytoplasm than adjacent cells not containing fungal structures. Distinction of symbiont respiration from increases in the respiration of host tissue itself is often difficult or impossible, however, and the two are often considered jointly as part of the overall carbon cost of infection. Changes in the size and nutritional status of mycorrhizal plants often complicate direct comparison and fertilizer supplements are sometimes necessary in nonmycorrhizal controls. Comparison of results is also often difficult because of different experimental species and conditions, but some general patterns emerge.

Many investigators have found increased allocation of C to mycorrhizal roots. This is often associated with greater respiratory losses and increased C fixation rates, which may or may not compensate for the increased carbon drain. Pang and Paul (1980) found additional allocation of C to mycorrhizal roots of *Vicia faba* equivalent to about 10% of total photosynthate. Nonmycorrhizal plants allocated 37% of fixed ^{14}C below ground whereas the corresponding figure for mycorrhizal plants was 47%. This difference was due to increased respiration from the roots of infected plants. Mycorrhizal plants had similar yields to uninfected ones, indicating that respiratory losses were compensated for by increased C fixation. Kucey and Paul (1982) found that N_2 -fixing *Rhizobium* nodules of mycorrhizal *V. faba* plants utilized 12% of total photosynthate, whereas those of nonmycorrhizal plants only used 6%. The C-fixation rate was also increased by 8% in plants supporting mycorrhizal symbionts. Snellgrove et al. (1982) also found that *Allium porrum* plants translocated 7% more C to mycorrhizal roots than to nonmycorrhizal roots of similar sized plants. The increased C allocation was associated with a decrease in specific leaf mass and increased leaf hydration. They suggested that this adaptation could enable mycorrhizal plants to maintain a greater photosynthetic capacity without increasing plant C requirements. Allocation of carbon to *Rhizobium* nodules of mycorrhizal *Glycine max* plants in experi-

ments by Harris et al. (1985) was increased to 12% of total photosynthate compared with 9% in nonmycorrhizal plants. Photosynthetic fixation rates were increased by up to 47% in plants with both symbionts, compared with nonsymbiotic, fertilizer-treated plants. Below ground CO₂ evolution was similarly increased from 9 to 29% in 6-week-old plants, the two symbionts together accounting for 82% this figure. In a recent, detailed, study Jakobsen and Rosendahl (1990) examined the distribution of ¹⁴C in the mycorrhizal plant–soil system of cucumber plants fed with ¹⁴CO₂. Control plants exhibited stress symptoms so detailed comparison of mycorrhizal and nonmycorrhizal plants was not possible, although total root activity and below ground respiration were five times higher in the mycorrhizal plants than the uninfected controls, representing 13.2 and 27%, respectively, of the total photoassimilated ¹⁴C. Altogether 43% of the total assimilated ¹⁴C was translocated to the root system and 70% of this was lost as CO₂ or extraradical C (exudates plus mycelium).

Estimation of the actual proportion of assimilate allocated to the mycorrhizal symbionts themselves is more difficult since it requires the separation of external mycelium from plant roots and soil, and necessitates assumptions concerning the efficiency of substrate incorporation. Nevertheless, strikingly consistent figures have been suggested. Kucey and Paul (1982) estimated that the mycorrhizal fungi of both nodulated and nonnodulated hosts utilized approximately 4% of the total C fixed by their hosts and that they constituted 5% of the root mass. Harris et al. (1985) estimated that the allocation to 6-week old plants infected with both *Glomus fasciculatum* and *Rhizobium japonicum* was 16.4% of the total assimilated carbon, of which 2.7% was attributed to biomass and 13.7% to respiration. These estimates were 2.8 and 4.6%, respectively, in 9-week-old plants, assuming an equal distribution of activity in intra- and extraradicle hyphae. In the study by Jakobsen and Rosendahl (1990) activity in the external hyphae represented 0.8% of total fixed ¹⁴C. These authors estimated total allocation to the external mycelium as 4% and total allocation to the internal hyphae as 16% (assuming a growth yield of 0.2 mg hyphal C per mg substrate C and assuming the internal infection to be 10% of root dry weight).

There are few studies which relate losses of C compounds via root exudation to assimilate allocation to external mycorrhizal mycelium. Kucey and Paul (1982) were unable to account for 3.2% of the ¹⁴C loss from roots, which could be accounted for by exudation. Jakobsen and Rosendahl (1990) noted that the activity of soluble and insoluble fractions of extraradical C in mycorrhizal systems was double that found in control systems and, together with the activity in external hyphae, represented 3.1% of total fixed ¹⁴C. The hyphae represented 26% of this total so that 2.3% of the total C fixation could be accounted for by exudation, a figure similar to those of Whipps and Lynch (1985). Losses from decomposition, leakage of the hyphae, and internal recycling of carbon compounds remain unquantified although their possible significance is discussed later in this chapter.

Studies of ectomycorrhizal carbon allocation show a similar pattern. Nelson (1964) demonstrated that allocation of carbon to mycorrhizal roots of *Pinus strobus* was almost four times that to nonmycorrhizal roots on a dry weight basis. Bevege et al. (1975) demonstrated an 8-fold increase in the roots of the same *Pinus radiata* plants infected with the ectomycorrhizal fungus *Rhizopogon luteolus* compared with nonmycorrhizal roots. More recent studies by Cairney et al. (1989) of carbon distribution within ectomycorrhizal root systems of *Eucalyptus pilularis* infected with *Pisolithus tinctorius* indicate that the amount of assimilate transferred to mycorrhizal roots is between 42.6 and 4.2 times that transferred to nonmycorrhizal root tips in the same root system. The same study showed that young roots acted as stronger sinks for activity and that there was a progressive reduction in translocation of photosynthate with age. In studies of carbon allocation in *Pinus taeda* and *Pinus contorta* infected with *Pisolithus tinctorius* and *Suillus granulatus* Reid et al. (1983) found that mycorrhizal plants assimilated more CO_2 , allocated a greater proportion to their root systems, and lost a greater percentage of ^{14}C by root respiration than did nonmycorrhizal plants.

Regulation of Carbon Flow

The relative importance of different processes regulating carbon flow to mycorrhizal roots is still not clear. It is apparent from the studies mentioned above that mycorrhizal infection is frequently associated with raised photosynthetic rates, increased carbon translocation to infected roots, and higher root respiration rates. Several explanations have been put forward. One suggestion is that irreversible conversion of plant assimilates to fungal-specific carbohydrates occurs (Lewis & Harley, 1965), creating a fungal sink that may indirectly increase the rate of photosynthesis. This is consistent with the idea that photosynthesis is under some control by sink demand. Mycorrhizal fungi may create such a fungal sink by converting carbohydrates to storage products, utilizing assimilates for fungal biomass production, or by using carbohydrates as energy for maintenance metabolism. Although labelled fungal carbohydrates can be measured, the latter two components of this sink have been difficult to quantify, especially with respect to the extramatrical mycelium. Other factors may also have a direct or indirect influence on photosynthesis and translocation. These include improved mineral nutrition, fungal-produced hormones, or changes in the balance of root hormones (Reid et al., 1983). Nylund and Wallander (1988) found circumstantial evidence of hormone effects, supporting the theory of Slankis (1973) that the mycobiont strongly affects its host via auxin action. In their experiments they grew *Pinus contorta* plants under steady-state nutrient conditions in semihydroponic culture. Auxin (IBA and IAA) treatments increased carbohydrate concentrations, but nutrients had no demonstrable effects on root or shoot carbohydrate contents. Free access to balanced, low concentrations of nutrients did not inhibit mycorrhiza formation and photosynthesis was considerably increased in mycorrhizal seed-

lings even though concentrations of N and Mg were similar in infected and uninfected plants. Growth of non-nutrient-limited mycorrhizal plants was reduced, suggesting that the mycorrhizal fungi imposed a carbon drain on their hosts, obtaining carbohydrates by means of an active process. This contradicts the notion that mycorrhizal fungi are simply the passive recipients of "surplus" plant carbohydrates (Björkman, 1942; see also Nylund, 1988). Similar reductions have been recorded in the growth of non-nutrient-limited plants infected with VA mycorrhizal fungi (Douds et al., 1988). Finlay (1989) speculated that, in certain situations, a deleterious carbon drain on the host plant may also result from infection by a poorly compatible ectomycorrhizal fungus. Studies of incompatible ectomycorrhizal associations between *Pinus sylvestris* and *Boletinus cavipes* (Finlay, 1989) demonstrated apparently normal translocation of ^{14}C -labeled assimilates to the fungal mycelium even though infection of incompatible hosts was poor and translocation of ^{32}P -labeled phosphate to them was severely reduced.

The cost-benefit relations of carbon drain, on one hand, versus growth stimulation through improved nutrient uptake, on the other, deserve further attention and are important when considering the "efficiency" of different mycobionts. Consideration of the relative size of structural and maintenance sinks has been complicated by the difficulty of measuring biomass and respiration of the external mycelial phase of mycorrhizal associations. The first studies of respiratory activity specific to the mycelial phase of ectomycorrhizal associations (Söderström & Read, 1987) demonstrated that approximately 30% of total respiration was due to that of the mycorrhizal mycelium. Mycelial respiration was shown to be highly dependent on the supply of current assimilate and severance of mycelial connections at the roots led to greater than 50% decreases in respiration rate within 24 hr. Such studies show the potential importance of the mycelial phase in carbon cycling.

Recent studies in our laboratory by Erland et al. (1991) show that up to 6% of assimilated carbon is present in the external mycelium of mycorrhizal *Pinus contorta* seedlings within 96 hr of supplying $^{14}\text{CO}_2$ to the shoot systems. In pulse labeling experiments the ^{14}C content of mycorrhizal roots was only 50% of that in nonmycorrhizal roots within 48 hr of supplying a 1-hr pulse of $^{14}\text{CO}_2$, although the relative amounts of C respired by the roots and the fungus could not be measured separately.

Mycorrhizal Mycelia

Methods of Measurement

Fungal biomass in and on the roots can be estimated by direct sampling and analysis of the roots: fruiting structures are often macroscopic and their biomass can be estimated. The mycelium growing in the soil is more difficult to quantify and our estimates of its biomass are therefore restricted. Much effort has been

put into the development of methods for estimating soil fungal biomass in general and a number of more or less imperfect methods exist that can be applied to mycorrhizal systems.

The most commonly used methods for measuring fungal mycelium in soil are still based on microscopic estimations of hyphal length, many of them based on modifications of the agar-film technique originally described by Jones and Molisson (1948). A somewhat simplified preparation technique was introduced by Hanssen et al. (1974) and their membrane filter method is now probably the most commonly used technique for direct microscopical estimation of hyphal length and width. Abbott et al. (1984) modified this technique for studies of vesicular-arbuscular mycorrhizal (VAM) external mycelium. One disadvantage of simple microscopical methods is that they do not distinguish between total biomass (including dead microbial cells), live biomass, and physiologically active biomass. Other methods, which distinguish these components, have been described, and one commonly used principle is to employ fluorogenic substances such as fluorescein diacetate (FDA) (Söderström, 1977). Each of these methods has specific drawbacks, but one common disadvantage is that distinguishing mycorrhizal hyphae from those of other fungi is not realistically possible.

Other methods of estimating fungal biomass that have been applied to mycorrhizal systems involve the analysis of fungal-specific metabolites such as ergosterol (Salmanowicz & Nylund, 1988) or chitin (Plassard et al., 1982; Whipps, 1987). These methods have been successfully applied to estimate fungal biomass, primarily within host tissue (Hepper, 1977; Bethlenfalvay et al., 1982; Vignon et al., 1986), but their efficacy in soil samples remains questionable as they do not differentiate mycorrhizal fungi from saprophytes or soil animals.

Analyses of respiration rates, or of substances that reflect microbial metabolic activity (ATP, enzyme activity), have often been used to provide relative biomass estimates. These indicators are of restricted use in mycorrhizal research, however, since they are totally unspecific and do not allow distinction of fungal activity, much less mycorrhizal activity.

The use of immunological methods provides a potentially more attractive approach in that these may allow distinction of specific mycorrhizal species. There is a vast literature available on the use of these techniques in medical mycology, but the application of immunological techniques to analysis of fungal systems in soil has been less successful. A number of qualitative studies have been published (e.g., Malajczuk et al., 1975; Aldwell et al., 1983, 1985; Wright et al., 1987; Frankland, 1975) but few attempts have so far been made to apply the technique quantitatively in mycorrhizal studies (Kough & Linderman, 1986). With wider use of DNA probes for identification of microorganisms (e.g., Festl et al., 1986; Stull et al., 1988) we can hopefully look forward to their application in mycorrhizal research.

A general problem in almost all microbial biomass estimations is the conversion of numbers of organisms, lengths of hyphae, or amounts of a specific measured

substance to actual biomass. The conversion factors used by different workers and in different soils vary greatly, restricting the general applicability of the methods.

Estimates of Mycorrhizal Mycelial Biomass

Vesicular-Arbuscular Mycorrhiza

The majority of measurements of mycorrhizal mycelium have been made in VAM systems. Measuring fungal biomass in a solid medium such as soil is difficult, and the problem becomes even worse when only a specific proportion, such as the external mycelium of mycorrhizal fungi, is to be estimated. Sanders et al. (1977) analyzed the fungal biomass gravimetrically, thereby avoiding all conversion problems. Using pure sand as a growth support they removed the mycelium attached to the root manually and weighed it to obtain a direct biomass estimate. They found 3.6 μg dry weight of mycelium per cm root using onion plants. In an earlier paper Sanders and Tinker (1973) calculated a length of 80 cm mycelium per cm of infected root (45 cm/cm total root) in onion plants inoculated with a *Glomus* species.

Other studies of external mycelium have been based on estimates of hyphal length. In two papers Abbott and Robson assessed the external mycelium of subterranean clover *Trifolium subterraneum*. In the first study (Abbott et al., 1984) they investigated the effect of phosphate supply on the development of external mycelium of *Glomus fasciculatum*. In control treatments they found 200 cm of hyphae per g of soil after 6 weeks growth. This corresponded to 450 cm of hyphae per cm of infected root. In phosphate treatments the corresponding values were 1523 cm/g soil and 900 cm/cm infected root. They did not attempt to estimate biomass, but assuming a hyphal diameter of 2 μm and a dry weight conversion factor of 0.2 (Bakken & Olsen, 1983), the control and phosphate treatment biomass would be 2.8 and 5.6 $\mu\text{g}/\text{cm}$ infected root, respectively, or 1.2 and 9.6 $\mu\text{g}/\text{g}$ fresh weight of plant root. However, it should be emphasized that such crude estimates can be used to estimate biomass only within an order of magnitude. If the mean hyphal diameter were set to 4 μm the estimated biomass figures would be 4 times higher.

In the second study, Abbott and Robson (1985) estimated amounts of external mycelium formed by four different endophytes with subterranean clover. In these experiments, *Glomus fasciculatum* produced only small amounts of external mycelium but *Glomus calospora* produced 200 cm/cm of hyphae per infected root after 4 weeks growth, increasing to 3000 cm/cm after 5 weeks and decreasing again to 1200 cm/cm after 7 weeks. *Glomus fasciculatum* produced 1400 cm/cm after 4 weeks and 300 and 200 cm/cm after 5 and 7 weeks growth, respectively. These authors also found that 30–50% of the mycelium in the pots inoculated with these two species was between 1 and 5 μm in diameter. These data illustrate

the complexity of biomass estimates and the need for repeated measurements at different time intervals. However, again assuming a mean hyphal diameter of 2 μm and 20% dry weight, the maximum and minimum biomass values in these studies would be 1.2 and 18.9 $\mu\text{g}/\text{cm}$, respectively. In a more recent study, Jakobsen and Rosendahl (1990) estimated that there was 27 m of external hyphae per g soil dry weight, constituting 2.6% of root dry weight.

Chitin analysis has mainly been used to estimate amounts of internal infection. Hepper (1977) showed that chitin measurements correlated well with amounts of infection and that the regression obtained differed significantly between different endophytes. She estimated the fungal biomass in *Centrosema pubescens* roots to be between 50 and 138 $\mu\text{g}/\text{mg}$ dry root in plants with high infection rates. Estimates of internal infection of *Trifolium repens* were as high as 17% root dry weight. The method has also been used to estimate soil VAM mycelial biomass (Pacovsky & Bethlenfalvay, 1982). Bethlenfalvay et al. (1982) found approximately 600 mg dry weight of intraradical fungal mycelium per plant using this method and about 100 mg of extraradical fungus.

Schubert et al. (1987) used FDA to estimate the viable proportion of the extramatrical mycelium in systems of *Trifolium repens* inoculated with *Glomus clarum*. As in other studies, the extraradical biomass increased during the growth of the plants reaching an apparent maximum after 8 weeks. Interestingly however, the FDA active proportion of the hyphal length decreased from 75–90% after 20 days to only 5–10% after 40 and 69 days in two different experiments. The low percentages seem to indicate that a stable equilibrium had been reached since in natural ecosystems the proportion of FDA stained mycelium is always rather low (Söderström, 1979). In another study, using iodonitrotetrazolium and NADH to estimate activity of external VAM hyphae, Sylvia (1988) found that the proportion of active hyphae in soil ranged between 0 and 32% of total length, but that the activity of hyphae attached to roots was much higher.

Ectomycorrhiza

Ectomycorrhizal mycelia are often extensive but estimates of mycelial length are fewer than for VA systems and this probably results from the fact that these fungi typically inhabit soils with a high humus content making the mycelium much more difficult to extract and visualize than in clay or sandy soils. Read and Boyd (1986) estimated hyphal lengths of between 10 and 80 cm/cm root length in laboratory systems containing *Pinus sylvestris* and *Siullus bovinus*, suggesting a similar order of magnitude to that in VA systems. The estimation of hyphal lengths in ectomycorrhizal systems is further complicated by the fact that the fungi often form differentiated structures in which hyphae aggregate to form mycelial strands. In contrast to studies of VAM fungi, which have often been carried out in pot cultures, most information on ectomycorrhizal fungi has come

from field studies where the aim has been to estimate the energy demands of the fungi.

Ericoid Mycorrhiza

Available evidence suggests that, in contrast to the above systems, ericoid mycorrhizal systems possess a poorly developed external mycelium (Read, 1984). Although the estimated number of entry points is high, ranging from 250 to 2000/cm root, the external mycelium only extends 0.5 to 1.0 cm from host roots. The ratio of external to internal fungal biomass may be as low as 0.1 (Read, 1984).

Energy Demands of Mycorrhizal Fungi

In contrast to VA mycorrhizal systems, where only 5–10% of the root weight is thought to be accounted for by internal infection, fungal tissue may constitute up to 40% of the dry weight of ectomycorrhizal roots (Harley & Smith, 1983). It is difficult to calculate an accurate biomass since the fungal mass will vary enormously between both different fungal species as well as host species. However, on the basis of such estimates, Fogel and Hunt (1979) calculated a fungal sheath biomass of 6104 kg/ha and a sclerotial biomass of 2158 kg/ha for *Cenococcum geophilum* in a *Pseudotsuga menziesii* forest in Oregon. They also measured the mycelium in the soil and estimated that 50% of the total stand throughput was accounted for by the fungi. In a later study (Fogel and Hunt, 1983) they reduced this figure to 28%. Vogt et al. (1982) estimated 15% of the net primary production in an *Abies amabilis* forest was consumed by mycorrhizal fungi. This study was based on the biomass of fine roots and it is possible to estimate a corresponding figure for Swedish pine forests. Persson (1978) estimated that fine (<2 mm in diameter) root biomass production in a young pine forest was 2030 kg/ha/year. Assuming that 90% of these tips are mycorrhizal and that 40% of the mycorrhizal tip weight is fungal, 730 kg of fungal sheath may be produced per hectare per year. The forest in which this production was estimated is a heathland pine forest where the dominant fruitbody-forming species is *Lactarius rufus*. There are no reliable data on fruitbody production in this forest but Richardson (1970) estimated the annual production of this species to be 265–460 kg fresh weight/ha. It is thus reasonable to assume a production in the Swedish forest of 30 kg/ha/year. There are no direct estimations of mycorrhizal mycelium in this forest, but Finlay and Söderström (1989) suggested a figure of 200 m/g dry weight on the basis of regression relationships between mycelial respiration and biomass. Using data from Söderström (1979) that figure can be converted to 3.5 kg live mycelium/ha dry weight standing crop. If a turnover rate of 1 week during the 5-month vegetation period is assumed the production will be 70 kg mycelium/ha/year. Together these estimates produce a total fungal production of 830 kg/ha/year. If the carbon content is set to 40% and the productivity to 60%, the carbon demand

of the ectomycorrhizal fungi in this forest will be 830 kg C/ha/year. The photosynthetic assimilation of the pines in this forest has been calculated to be 5800 kg C/ha/year (Linder & Axelsson, 1982), which means that the ectomycorrhizal fungi, according to these calculations, will use 14–15% of the assimilated carbon, a figure that is strikingly similar to the ones presented by Vogt et al. (1982), Fogel and Hunt (1979), and also similar to estimates of carbon flow to VA mycorrhiza.

Regulation and Adaptation

Mycorrhizal mycelia are dynamic systems, but we still know rather little about the processes that regulate their behaviour. These can be considered on two levels. First, regulation of mycelial growth may be brought about by short-term effects such as changes in soil conditions and light or plant-induced changes. Second, adaptive changes in mycelial structure and function in response to different environments also occur over evolutionary time.

In experiments by Piché and Fortin (1982) the addition of increasing amounts of ammonium (0–2 mg N per seedling) to growth pouches containing *Pinus strobus* improved the development of mycelial strands, whereas increases in phosphorus concentration caused reduced mycorrhizal infection and poorer mycelial development. Reduced light intensities also reduced mycelial development, showing the importance of current assimilate for mycelial biomass production. Little is known about the longevity and turnover of mycorrhizal roots and mycelia and further experimentation in this area is required.

The adaptive features of different mycorrhizal types that have evolved in response to different environments have been elegantly reviewed by Read (1984, 1987). It is noteworthy from the above discussion that the external mycelium of ericoid mycorrhizae is poorly developed compared with VA and ectomycorrhizas. Read (1984) has pointed out that, owing to the high water-holding capacity of mor humus, the roots of ericaceous heathland plants are normally bathed in a dilute solution of carboxylic acids containing free and adsorbed nutrients. Under such conditions the production of an extensive external mycelium would be a wasteful investment of carbon since no nutritional benefit would arise. Investment of carbon in external mycelia is generally higher in ectomycorrhizal and VA mycorrhizal systems but differences do occur. The transition from the mor humus of boreal coniferous forests to the mull humus of temperate deciduous forests is often associated with a general trend toward decreased production of ectomycorrhizal external mycelium. It has been suggested that efficient absorption and storage of leached nutrients in a thick sheath may be more important in environments where there are seasonal flushes of nutrient release from fallen litter, whereas the maintenance of an extensive mycelium may be better adapted to the acquisition of permanently low levels of mineral nutrients (Read, 1984; Finlay

et al., 1989). The consequences of these two strategies in terms of carbon flow remain open to speculation.

Information about the dynamics and turnover of mycorrhizal mycelia in natural soil ecosystems is still severely limited. In the following section we consider the consequences of carbon flow to and through mycorrhizal mycelia in terms of its effects on the soil community.

Consequences of Carbon Flow to Mycorrhizal Mycelia: Effects on the Soil Community

It is clear from the preceding discussion that significant amounts of energy rich carbon compounds are supplied directly to the mycorrhizal mycelium in a range of associations. Estimates of the amounts of carbon involved vary but it seems likely that in many soils the mycelia of VA and ectomycorrhizal associations may form a significant proportion of the total fungal biomass. Discussion of the ecological significance of this mycelial phase has, until recently, been restricted to the consideration of ways in which the supply of mineral nutrients to individual plants or roots is ameliorated. However, the significance of mycorrhizal mycelia has recently been considered in the wider context of its possible influence at the community and ecosystem levels (Read, 1984; Read et al., 1985; Finlay & Söderström, 1989). Some of these wider roles are discussed in other chapters. Here we consider these effects in relation to the consequences of carbon input through mycorrhizal mycelia to the soil ecosystem.

Utilization of Simple and Polymeric Organic Compounds

Evidence concerning the degree of hydrolytic activity of different mycorrhizal fungi and its possible significance in different ecosystems is still incomplete. However, the supply of energy-rich carbon compounds for the synthesis of hydrolytic enzymes is clearly of potential importance. The effects of possible activity on nutrient cycling and interactions with the saprophytic microflora are potentially great in terms of overall mineralization processes and microbial immobilization.

The proteolytic capability of different mycorrhizal fungi differs greatly and more detailed studies are required to assess its importance. Abuzinadah et al. (1986) suggested that direct utilization of organic nitrogen sources by ectomycorrhizal fungi would lead to tighter nutrient cycling by restricting losses to decomposer populations. These authors suggested an alternative view of the forest nitrogen cycle based on proteolytic and peptidolytic capability in some ectomycorrhizal fungi. Mycorrhizal fungi are unique in that they have direct access to a supply of energy-rich substrates in the form of plant assimilates, and this may place them at a competitive advantage with respect to the saprophytic microflora. Finlay and Read (1986a) demonstrated that distribution and activity of ectomycor-

rhizal mycelia were not uniform and speculated that the mycelium may be capable of selective exploitation of localized sites of nutrient enrichment, in a manner similar to that suggested for VAM fungi by St. John et al. (1983). Finlay and Söderström (1989) pointed out that selective colonization of areas of soil rich in organic material would be of particular importance in species with proteolytic capability. The mycelial patches found by Finlay and Read (1986a; 1986b) were strong sinks for both labelled plant assimilates and phosphorus, and the ability to direct carbon compounds required for the synthesis and excretion of protease enzymes, selectively to sites of organic enrichment would clearly be advantageous.

Other organic compounds containing carbon can be used by mycorrhizal fungi and their use as carbon sources may affect the carbon economy of the symbiosis. Trojanowski et al. (1984) suggested that ectomycorrhizal fungi might be able to utilize lignin but experiments by Haselwandter et al. (1987) showed that the ability of the ectomycorrhizal fungus *Paxillus involutus* was low compared with two ericoid endophytes. Other recalcitrant polymers such as tannic acid can also be degraded by the ericoid endophyte *Hymenoscyphus ericae* (Leake & Read, 1989) and soluble, phytotoxic phenolic acids can also be used as carbon sources. The utilization of organic compounds in the above ways should reduce the carbon drain imposed on the host plant by the mycobiont.

Translocation and Uptake of Mineral Nutrients

The allocation of carbon to mycorrhizal mycelia for biomass production and energy requirements is of fundamental significance to processes of mineral nutrient uptake but is not mentioned further here since this is fully discussed in the previous chapter (Chapter 4, Read).

Mycelial Connections between Plants

One consequence of the extensive growth of external mycorrhizal mycelium and the generally low host specificity of mycorrhizal fungi is that inter- and intra-specific mycelial connections are formed between adjacently growing plants. The full ecological significance of these is still uncertain. Evidence from isotope studies suggests that compounds containing C, N or P can move between plants through these connections (Read et al., 1985; Francis et al., 1986; Finlay and Read, 1986a; Haystead et al., 1988). However, the question of whether net transfer actually occurs and, if so, whether the quantities involved are significant is still a matter of some controversy (see Newman, 1988).

There is presently little evidence to suggest that such movement of compounds is quantitatively important compared with "normal" uptake of phosphate or ammonium, or with photosynthetic C fixation. The transfer of amino acids and amides across the host-fungus interface (Finlay et al., 1988, 1989) allows for bidirec-

tional movement of C in addition to the net carbohydrate flux required for fungal growth. Direct evidence for this reverse flux has been provided by Duddridge et al. (1988). However, the integration of plants into a common mycelial network may be of significance without necessarily implying a large net reverse carbon flux. The capacity for lateral movement of host-derived assimilates along chemical concentration gradients from areas of high substrate availability to areas of low substrate availability, as demonstrated by Francis and Read (1984) and Finlay and Read (1986a), will allow small seedlings to become connected to a much larger mycelium than they could support on the basis of their own photosynthetic products alone. Even small reductions in the carbon drain from small, shaded seedlings by virtue of "lateral" transport of assimilate from larger, better illuminated plants may be of significance to plants at or near their compensation point, and important in processes of regeneration, without implying a large net flux of carbon from fungus to plant. Additionally, as Finlay and Read (1986b) pointed out, seedlings with small root systems and limited seed reserves become connected to a mycelium, which can exploit a larger volume of soil for mineral nutrients than would be possible solely on the basis of allocation of their own assimilates. Again, no arguments of net interplant transfer need to be invoked for this to be so.

Unequivocal demonstration of these processes requires further experimentation, but a number of observations lend support to the idea. Experiments by Fleming (1984) demonstrated that ectomycorrhizal infection of seedlings was reduced in cored or trenching areas suggesting that mycelial connections with mature trees were important for the successful establishment of infection. Recent studies using experimental microcosms (Grime et al., 1987), or manipulated field systems (Gange et al., 1990), also suggest that the presence of vesicular-arbuscular mycelial connections may have important effects on community development, influencing the survivorship and competitive ability of particular species and promoting species diversity. Less experimental evidence exists for ectomycorrhizal systems, but recent experiments by Perry et al. (1989) have provided tentative evidence that ectomycorrhizal fungi may mediate competition between different host species. Ultimate effects of mycelial connections on species diversity may depend on the degree of host specificity existing in the ecosystems under consideration, as pointed out by Finlay (1989).

Aggregation of Sandy Soil

Another consequence of the extensive production of external mycelium by mycorrhizal fungi is that the hyphae help in binding soil particles (Sutton & Sheppard, 1976) to form stable soil aggregates. This may reduce soil erosion and water runoff and be particularly important in loose mine soils (Rothwell, 1984). Tisdall and Oades (1979) recorded mycelial lengths of up to 55 m/g of soil associated with grassland species.

Grazing by Animals

It is clear from the preceding discussion of mycorrhizal mycelial biomass that in many situations it may represent a significant proportion of the total soil mycelial biomass and thus represent a significant resource to the mycophagous soil fauna. The subject of grazing interactions between soil fauna and mycorrhizal fungi is dealt with more fully elsewhere in this volume (Fitter & Sanders, Chapter 10) and is mentioned here only in so far as the cycling of carbon compounds is involved. Many previous studies of the effects of soil have been restricted to the overall effects of grazing on nutrient cycling and there have been few specific studies of the direct effects of grazing of mycorrhizal mycelia (Finlay, 1985; Shaw, 1985; Mcgonigle & Fitter, 1987). More studies are required of interactions between mycophagous soil animals and mycorrhizal fungi to determine the extent of grazing and its possible effect on plant growth and mycorrhizal nutrient cycling. One possible use of carbon compounds transferred to mycorrhizal hyphae is the production of secondary compounds to inhibit grazing.

Mycorrhizosphere Effects

The term "mycorrhizosphere" was suggested by Rambelli (1973) to describe the soil surrounding, and influenced by, mycorrhizas. Evidence for the existence and possible significance of mycorrhizosphere effects has been reviewed by a number of authors (e.g., Fogel, 1988; Linderman, 1988) and will be discussed later in this book (Azcon-Aquilar & Barea, Chapter 6).

A key feature of the mycorrhizosphere is the presence of mycorrhizal hyphae that surround the root and extend out from it in the form of dense extramatrical hyphae. These hyphae may extend the limits of the mycorrhizosphere considerably past those of the normal rhizosphere of uninfected roots. Interactions with other soil microorganisms may be direct or indirect, through effects on the host plant. Mycorrhizal infection may cause changes in the quality and quantity of root exudates and secretions, enhance the nutrient and elemental composition, alter the hormone balance of host roots, and increase respiratory losses of CO₂ from the root surface. Since mycorrhizal hyphae often dramatically influence the distribution and absorptive surface area of root systems they present a considerable surface area across which direct interactions may take place with the microbial flora. A "mycosphere" may thus develop around mycorrhizal hyphae in which enhanced microbial populations of altered species composition may occur.

The extramatrical hyphae themselves may exude substances that cause soil and organic matter to aggregate (Sutton & Sheppard, 1976) providing microsites for growth of bacteria, fungi, actinomycetes, and algae. In cases in which the root is more or less completely surrounded by fungal material, such as the sheathed lateral roots of ectomycorrhizal plants, most or all of the substances entering the soil may do so through fungal hyphae. Interactions may be stimulatory, inhibitory,

or neutral (Bowen & Thoedorou, 1979). Stimulatory effects of microorganisms on ectomycorrhizal colonization and development have been reported by Garbaye and Bowen (1989) and MacAfee and Fortin (1988). The *in vitro* growth of different ectomycorrhizal isolates can also be both stimulated or inhibited by actinomycetes isolated from the mycorrhizoplane of *Pinus resinosa* (Richter et al., 1989). It seems that distinct microbial communities may have evolved in response to the presence of specific mycorrhizal associations, and the isolation and selection of the appropriate "helper" organism, for use as coinoculants, may offer scope for additive or synergistic growth stimulation.

While exudation of specific compounds from the roots or mycelium may stimulate microorganisms beneficial to the symbiosis, extracellular metabolites may also have an antibiotic effect on certain phytopathogenic microorganisms (Kope & Fortin, 1989). The continuous supply of carbohydrates that mycorrhizal fungi receive from their hosts is probably important in terms of providing the energy for synthesis of the wide range of compounds that are undoubtedly involved in these microbial interactions. It is often assumed that mycorrhizal fungi are at a competitive advantage with respect to the saprophytic flora because of this direct supply of plant assimilates. Interactions with saprophytic populations could thus influence decomposition processes. Abuzinadah et al. (1986) suggested that the increased rates of pine litter decomposition following exclusion of ectomycorrhizal roots (see Gadgil & Gadgil, 1971, 1975) could be caused by the removal of successful competition for limited organic nitrogen by ectomycorrhizal fungi with proteolytic enzymes. Death, decomposition, and leakage of organic compounds from decomposing mycorrhizal hyphae represent a potential input of carbon into the soil ecosystem about which we know very little. Further information about their dynamics and turnover is required. However, one consequence of the proteolytic activity of certain ectomycorrhizal species is that organic compounds released from dying hyphae could be used directly by living mycorrhizal hyphae and the carbon recycled internally within the ectomycorrhizal association, restricting losses to the soil ecosystem through immobilization.

Concluding Remarks

Carbon flow to mycorrhizal roots and through mycorrhizal mycelia to different components of the soil ecosystem can clearly be significant. In forests it has been estimated that 3–5 times more organic matter is returned to the soil in the form of roots and mycorrhiza than is returned by decomposition of litter (Fogel, 1988). Data from many different sources now suggest that as much as 20% of the total carbon assimilated may be transferred to mycorrhizal fungi in both VA and ectomycorrhizal systems. Fewer data exist for ericoid mycorrhizal systems where the production of external mycelium is less extensive. More information is needed about the amounts and types of carbon compounds involved and the processes

that regulate their translocation to mycorrhizal roots and flow through the mycelium. Large gaps remain in our knowledge and further information about these important processes is needed, not least because the flow of carbon to mycorrhizal symbionts under different conditions may have important economic implications to crop and timber yields.

The available methods for quantifying the total and active proportion of the external mycorrhizal mycelium are still inadequate and need further development. Until these are improved, progress in understanding the dynamics of mycorrhizal mycelia and their interactions with other organisms will be restricted. For this reason, concepts of mycorrhizal efficiency, involving the balance between carbon drain and the beneficial effects of increased mineral nutrient uptake, are still poorly developed.

The flow of carbon to mycorrhizal mycelia clearly has a potentially huge range of effects since energy-rich substrates are required by most biological processes. Potential effects include interactions with both phytopathogens and decomposers, chemical defences against grazing of the mycelium, and the stabilization of soil aggregates. These may have an impact on energy flow and cycling of nutrients. Wider effects on plant communities may also occur through influence on regeneration processes and plant competition.

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